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13. ABSTRACT (Maximum 200 words)

The objectives of this work have been to develop a better understanding of the physiology and functional organization of the auditory periphery in fishes and to determine their ability to perform sound source localization. These results can potentially be applied to developing better computational models to help elucidate the methods by which fishes detect and process low-frequency acoustic signals. Emphasis in our work was placed upon mechanisms of sound source localization including the determination of the azimuth, elevation, and range of sound sources.

Our studies have involved determination of the anatomy of the goby ear, measurement of the response properties of single units from the goby ear and correlating responses with the morphology and directional orientation of the hair cells they innervate, and doing behavioral studies to determine the response sensitivity of the fish to sounds from different directions.

We have developed intracellular recording techniques which we are coupling with confocal microscopy to very specifically correlate response characteristics of single neurons with the directional orientation of hair cells and the number of hair cells that are innervated.

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Sound Localization by Fish: Mechanisms and Models

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OBJECTIVES

The mechanism(s) used by fish to determine sound source direction and distance has been enigmatic for most of the 20th century. Initially, it was not clear whether fish could localize sound. Not only was it difficult to determine localization capabilities due to the complexities of underwater sound, but investigators in the 1960's and 1970's were convinced that fish could not do determine sound source direction since the cues used for localization by terrestrial animals were not available to fish (interaural differences in time of arrival and intensity). In essence, investigators made the assumption (now known to be erroneous) that the only way to do sound localization is to compare inputs to two ears. Since the speed of sound in water is five times that in air, and since the two ears of fish are very close to one another (often within millimeters), the magnitude of time of arrival or amplitude differences would be so small as to not be discriminatable by the nervous system.

However, once investigators realized that fishes might be using methods to determine sound source direction that could be very different from the terrestrial model, new avenues of approaches to the problem opened up. Most significantly, it became apparent from behavioral and anatomical work that localization may involve processing of signal displacement by the ear itself instead of their being a binaural comparisons of signal differences from the two ears. The hypothesis was proposed that the sensory receptors of the ear, hair cells, were responsive to the vectorial components of particle displacement signals. The extrapolation from this hypothesis is that the fish ear functions as an exquisitely biological accelerometer that is potentially capable of resolving directional information in three dimensions.

Still, despite knowing that fish could localize sound, and that the ears were involved, earlier studies had not isolated the specific features of the ear that might be involved in localization. Moreover, it was clear whether the ear was 'wired' to take advantage of particle displacement information in an impinging sound wave and thereby carrying directional information to the brain. Thus, it became necessary to carefully quantify directional responses of the ear and to correlate

these responses with anatomy. Moreover, it was necessary to get careful behavioral measures of directional response capabilities of the ear.

The objectives of this work have been to develop a better understanding of the physiology and functional organization of the auditory periphery in fishes and to determine their ability to perform sound source localization. These results can potentially be applied to developing better computational models to help elucidate the methods by which fishes detect and process low-frequency acoustic signals. Emphasis in our work was placed upon mechanisms of sound source localization including the determination of the azimuth, elevation, and range of sound sources. We developed methods that allowed us to obtain the first data on directional responses of fully characterized neurons and then apply this information to developing new models for understanding how fish can determine sound source direction and distance.

APPROACH

Physiological and behavioral responses of several teleost species were investigated using a three-dimensional shaker system that provided directional stimulation to simulate underwater acoustic particle motion (e.g., Lu et al., 1996). This method enabled us to mimic the particle displacement signals normally imagining upon a fish in a sound field, but without having to provide for large open expanses of water that are needed in order to get a calibratable sound field.

Using this technique, we were able to do physiological studies to record single units (neurons) of the auditory portion of the eighth cranial nerve (intracellular and extracellular) (e.g., Lu et al., in press). In behavioral studies, fish are trained, using classical conditioning of heart rate, to respond when they detect a directional stimulus.

ACCOMPLISHMENTS

Initially we investigated localization in the oscar, *Astronotus ocellatus,* and determined behavioral sensitivity to stimuli from different directions (Lu et al., 1996). We demonstrated that the oscar was extremely sensitive to directional stimuli from all directions.

In later studies we found that physiological results could be more readily obtained using a different species, the sleeper goby, *Dormitator latifrons*. This goby is very amenable to physiological (and behavioral) studies since its ear is much more accessible than in the oscar and the fish is much more amenable to analysis of the relationship between the structure of the ear, and the physiological responses of single neurons. Accordingly, our studies have involved determination of the anatomy of the goby ear, measurement of the response properties of single units from the goby ear and correlating responses with the morphology and directional orientation of the hair cells they innervate, and doing behavioral studies to determine the response sensitivity of the fish to sounds from different directions.

Our most recent studies have resulted in the development of intracellular recording techniques which we are coupling with confocal microscopy to very specifically correlate response characteristics of single neurons with the directional orientation of hair cells and the number of hair cells that are innervated (Lu and Popper 1997).

A. Anatomy of the Goby Ear

We determined the structure of the goby ear using light and scanning electron microscopy.

The three otolithic organs (saccule, utricle, and lagena) of the ear each contain a sensory epithelium (macula) which contains large numbers of sensory hair cells. The macula is overlain by a very dense calcareous otolith. In accelerometer terms, the epithelium serves as a sensor and the otolith as the overlying mass.

Analysis of the epithelia demonstrated that each has a different spatial orientation from one another. The saccule is oriented perpendicular to the fish's horizontal plane and deviates about 40° from the mid-sagittal plane. The utricle lies on the horizontal plane, and the lagena is oriented vertically and about parallel to the mid-sagittal plane. The otolithic organs thus forms a 3-D acceleration detector which provides the structural basis for fish to perform sound localization. We hypothesized that the response directionality of fish auditory afferents results from the morphological polarity of sensory hair cells in otolithic organs.

B. Encoding of Acoustic Particle Motion by Saccular Afferents

Since it is difficult to compare the polarity of hair cells and neural response directionality in three dimensional space, we conducted initial experiments on the horizontal plane. As the saccular epithelium is oriented in a vertical plane that deviates about 40° from the midsagittal plane, the polarity of most hair cells projected on the horizontal plane is predicted along 30 to 60° off the longitudinal axis.

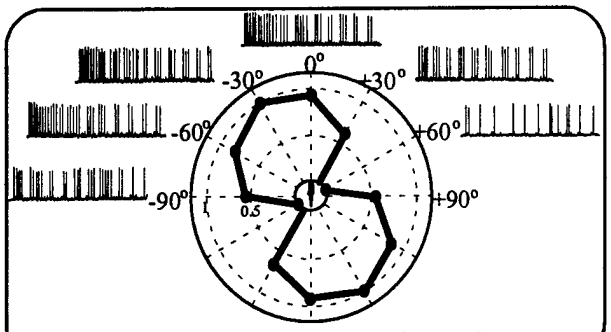

Figure 1: Directional response of a single neuron

In these experiments, recordings were made from a single afferent neuron as the fish was stimulated at various directions around the horizontal axis. The number of action potentials (spikes) were determined along each angle, and the best response axis (BRA) was then obtained. Figure 1 shows an example of responses of an afferent fiber in response to stimuli from different directions. These results show that this neuron is most responsive to signals that are from 30 degrees to the left of the fish.

Over the course of the experiments, we have extracelluarly recorded neural response from 107 single auditory nerve fibers in response to 100 Hz sinusoidal displacement at six pre-selected axes in azimuth and the vertical axis. The number of discharges and associated spike times were recorded to construct spike vs. angle functions and temporal response patterns (peri-stimulus-time histogram, PSTH; and period histogram). Almost all afferents were phase-locked to sinusoidal waveforms and showed one-peaked period histograms. In addition, a few afferents recorded had period histograms with two preferred peaks separated at about 180°, suggesting some auditory nerve fibers innervate hair cells with opposite orientations. In general, the axis along which a sound wave is propagating

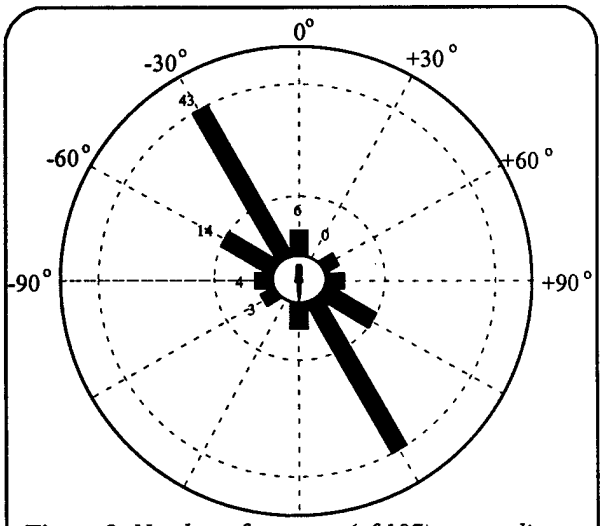

Figure 2: Number of neurons (of 107) responding to signals from different directions.

is encoded by the number of discharges of fish's auditory nerve fibers. Intracellular injection of Neurobiotin will be conducted to show the relationship between best response axes and morphological polarizations of hair cells innervated by physiologically characterized afferent fibers. We expect best response axes of fibers are correlated with hair cell orientations.

Figure 2 shows a polar plot of the distribution of best response axes for 80 afferent neurons. The results show that the vast majority of the neurons are responsive to signals provided along 30 degrees to the left of the animal. Correlation of these results with anatomical data show that the majority of hair cells in the left ear (the ear in which recordings were made) are oriented at the 30 degree angle. Thus, the orientation of the hair cells is directly correlated with the response properties of the neurons by which they are innervated .

C. Behavioral Studies

To find out the frequency range of hearing for the goby, fish were trained to respond to tones (100, 200 350, and 600 Hz) provided by an underwater speaker (Lu and Popper, in prep.). A behavioral audiogram obtained from a goby (see Fig. 3) indicates that the goby was most sensitive to 100-Hz tones. In addition, threshold dramatically increased as the stimulus frequency was raised. The sensitivity at 350 Hz decreases about 80 dB in reference to that at 100 Hz (dB re: the threshold at 100 Hz). It appears that the goby's swimbladder does not play important role in hearing. Furthermore, these results demonstrate that the goby is most sensitive to the particle motion component of the sound field.

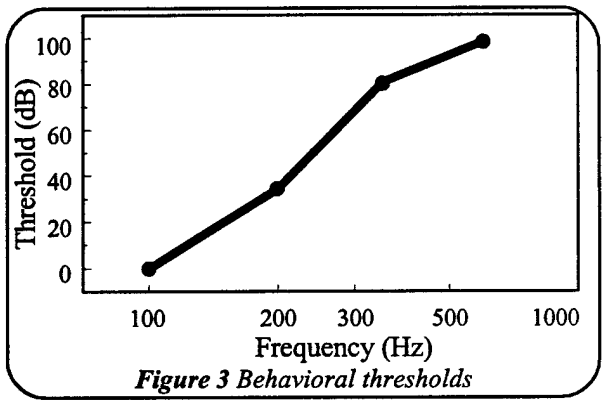

Figure 3 Behavioral thresholds

SIGNIFICANCE

We have developed a unique technique that enables us to obtain both physiological and behavioral data on sound source localization by fish in a single setup, thus providing us with the ability to measure behavioral and physiological aspects of sound source localization without having to resort to very large tanks. Our data provide physiological data on the response of the ear of a non-specialist fish, the oscar, to a directional stimulus. The behavioral data are the first ever obtained that provide a detailed analysis of directional sensitivity of any fish species. Our behavioral data have demonstrated that at least one species of fish is able to detect particle motion in the acoustic far-field with an inner ear sensitivity that rivals that of the mammalian organ of Corti. Significantly, the behavioral sensitivity results are in the same threshold range as that of individual neurons recorded both in the oscar and in a hearing specialist.

Our results demonstrate, for the first time, a clear correlation between hair cell orientation patterns in the saccule of a fish and the physiological responses of innervating neurons. These findings strongly support the hypothesis that the organization of the ear is directly responsible for determining particle motion direction, and that this information is sent on to the brain to provide direct information on sound source direction.

Most recent results, using intracellular filling of single neurons combined with confocal microscopy demonstrate that we should be able to correlate orientation patterns of individual sensory hair cells and the neurons that innervate them. In other words, we anticipate being able to

determine the specific input to individual neurons and correlated this directional information with the response properties of neurons. Ultimately, we will be able to use this method to trace individual neurons to the CNS and help understand the way directional information in coded in the brain.

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